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CLINICAL COUNTERPOINT: Vitamin D: New Actions, New Analogs, New Therapeutic Potential*

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I. Introduction

AN EXCITING new era has developed in the vitamin D field with the discovery of new target tissues, mechanisms of action, and selective analogs. As a clinical counterpoint article, this review will emphasize the potential clinical applications of these new discoveries. Many of the applications to be discussed remain untested, whereas others remain in their infancy. A major reason for optimism in predicting an expanding list of therapeutic applications for the vitamin D metabolites and analogs stems from the development of analogs of calcitriol that differ in their biological effects. In particular, analogs are now available that appear to separate the effects of calcitriol on growth and differentiation from effects on intestinal calcium absorption or bone mobilization. This apparent selectivity may reflect altered pharmacokinetic properties or may involve mechanistic differences at the cellular level as well. Regardless, the development of these drugs is likely to lead to clinical applications in which raising serum calcium need not accompany other actions. The initial portion of this review is devoted to a discussion of these analogs. The main goal of this article is to review those areas in which calcitriol and its analogs are being used in new ways and to describe potential applications that are suggested by the newly discovered actions of calcitriol. Thus, new concepts in vitamin D action have led to clinical trials of calcitriol and its analogs in the management of hyperparathyroidism and psoriasis, and trials of these drugs in certain malignancies or immunological disorders may not be far off. None of the clinical applications discussed are approved applications for these drugs. Rather, this review is intended to link the laboratory observations of the past decade with the bedside of the next decade.

In the past decade, a number of tissues have been found to contain receptors for the active vitamin D metabolite, $1,25(OH)_2D$, and to respond to this hormone with a change in function. The classic target tissues, bone, kidney, and intestine, responsible for maintaining bone mineral homeostasis in response to vitamin D and its metabolites are now only part of a list that includes several dozen tissues including various elements of the hematopoietic and immune system, cardiac, skeletal, and

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smooth muscle, brain, liver, breast, endothelium, skin (keratinocytes, melanocytes, and fibroblasts), and endocrine glands such as the pituitary, parathyroid gland, pancreatic islets (β-cells), adrenal cortex and medulla, thyroid, ovary, and testis. Furthermore, malignancies developing within these tissues may also contain vitamin D receptors (VDR) and be expected to respond to $1,25(OH)_2D$. The responses of these tissues to $1,25(OH)_2D$ are as varied as the tissues themselves. $1,25(OH)_2D$ regulates hormone production and secretion including insulin from the pancreas, PRL from the pituitary, and PTH from the parathyroid gland just as it regulates cytokine production and secretion such as interleukin-2 (IL-2) from the lymphocyte and tumor necrosis factor from the monocyte. Myocardial contractility and vascular tone are modulated by $1,25(OH)_2D$ as is liver regeneration. $1,25(OH)_2D$ reduces the rate of proliferation of many cell lines including normal keratinocytes, fibroblasts, lymphocytes, and thymocytes as well as abnormal cells of mammary, skeletal, intestinal, lymphatic, and myeloid origin. Differentiation of numerous normal cell types including keratinocytes, lymphocytes, hematopoietic cells, intestinal epithelial cells, osteoblasts, and osteoclasts as well as abnormal cells of the same lineage is enhanced by $1,25(OH)_2D$. Thus, the potential for manipulating a vast array of physiological and pathological processes with vitamin D-related compounds is enormous.

The major problem facing the clinician desiring to manipulate any one of these newly recognized actions of vitamin D is that $1,25(OH)_2D$ is likely to require higher than physiological doses to be effective and will not be selective at such doses. Thus, to use $1,25(OH)_2D$ to treat diabetes mellitus, control psoriasis, or modulate the growth of the tumor is to risk complications associated with hypercalcemia and hypercalciuria. Developing analogs of $1,25(OH)_2D$ to improve the selectivity and confer a lower risk- benefit ratio has become a major effort by several pharmaceutical firms, and the early results look quite promising. In a summary of their experience with 228 analogs of vitamin D, Norman *et al.* (1) have suggested that different analogs bind to the VDR from different tissues with different affinities (although this may be a species rather than tissue difference), that the affinity of an analog for the VDR in cells does not necessarily parallel its affinity for the circulating vitamin D binding protein (DBP), and that the affinity of an analog for DBP or VDR does not necessarily predict its biological activity. As will be discussed in more detail below, different cells respond differently to the various analogs, and, indeed, some of these analogs are being used in clinical trials for conditions such as psoriasis without apparent risk of hypercalcemia.

In this clinical counterpoint to Marian Walters' extensive review of newly identified actions of the vitamin D endocrine system (2) I will review recent progress in the development of vitamin D analogs that show clinical promise before examining clinical conditions in which these analogs or $1,25(OH)_2D$ (calcitriol) itself have been used or could conceivably be used in the near future. Most of the conditions that will be discussed are not approved indications for the use of calcitriol, and none of the analogs to be discussed have been approved for clinical use. Nevertheless, the trends are clear as we move into a new era in the therapeutic application of vitamin D metabolites and analogs. In this discussion calcitriol and $1,25(OH)_2D$ will be used interchangeably.

II. Promising Analogs

A: Natural metabolism

Ever since the discovery that vitamin D required metabolism first to 25- hydroxyvitamin D (25OHD) and then to a variety of metabolites, the most important of which is $1,25(OH)_2D$, considerable effort has been expended in determining the structure-function relationships of this family of vitamin D seco-steroids. The early work concerning these structure-function relationships has been reviewed previously (3, 4). The salient features are as follows. The most potent naturally occurring vitamin D seco-steroid in terms of stimulating intestinal calcium transport, mobilizing calcium from bone, raising serum calcium, and healing rickets (the "classic" actions of vitamin D) is 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). As depicted in Fig. 1, this molecule has three hydroxyl groups, two of which are in the A ring on opposite sides of the plane of the ring (3β-OH and 1α-OH) and the third in the side chain (25 OH). The 1α- and 25 OH

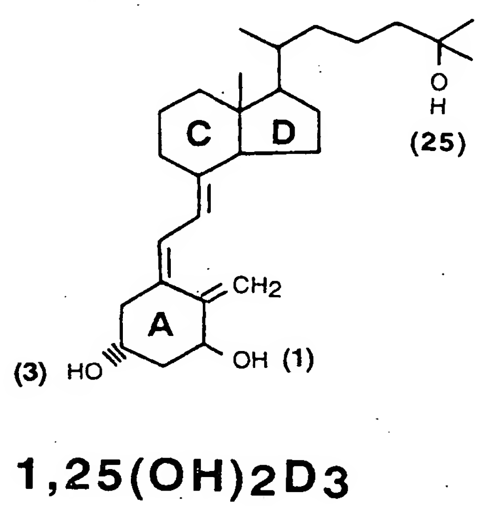

FIG. 1. The structure of $1,25(OH)_2D$ (CT). The three hydroxyl groups are numbered as to position as are the ring structures.

groups are critical for binding to the VDR. Removal of either OH group [as in 1α-OH D_3 or 25-hydroxyvitamin D_3 (25OHD_3)] reduces the affinity of the molecule for the VDR by approximately 3 orders of magnitude, and removal of both (as in vitamin D_3 itself) essentially eliminates binding to the VDR. In contrast, DBP has a higher affinity for 25OHD_3 than for either 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] or vitamin D_3 (5). Correlating with their importance for binding to the VDR, the 1α and 25 OH groups are required for full biological activity at least in terms of their classic actions in regulating calcium homeostasis. The 3β OH group is of lesser importance. Removing the 3β OH group reduces both affinity for the VDR and biological activity by less than 1 order of magnitude (6). The triene structure linking ring A and ring C is in the cis configuration in the naturally occurring metabolites. Rotation of ring A into the transconfiguration brings the 3β-OH into a pseudo 1α position. The presence of the pseudo 1α-OH in dihydrotachysterol (DHT) accounts for its greater biological activity compared to vitamin D in nephrectomized animals or patients with chronic renal failure who have lost their capacity for 1α-hydroxylation of the vitamin D seco-steroids. DHT is an early example of a clinically useful analog. 25-Hydroxy-DHT_3 (25OHDHT_3) binds to the VDR with 10 times higher affinity than 25OHD_3 and is the presumed biologically active metabolite of DHT (7). The side chain is the site of extensive modification by the body as well as by organic chemists (as will be discussed below). The 24 position is hydroxylated by many tissues, the OH group being inserted into the R position. Although the biological importance of 24,25$(OH)_2$D remains in dispute, the insertion of the 24(R) OH group into 1,25$(OH)_2D_3$ reduces its classic biological activity and affinity for the VDR (8, 9). Although the insertion of 24 OH adjacent to 25 OH in the side chain reduces the biological activity, 24 OH can substitute for 25 OH with little loss of activity (10). In other words, for optimal activity either a 24 or a 25 OH group is needed, but not both. The C23 and C26 positions also undergo hydroxylation, metabolic changes that reduce the biological potency of the parent molecule and appear to be part of the catabolic pathway for the active vitamin D metabolites (reviewed in Ref. 11). However, evidence has been obtained suggesting that 25,26$(OH)_2D_3$ stimulates intestinal calcium transport without raising serum calcium (12), making this metabolite an early example of a vitamin D compound with selective biological function.

B. Synthetic modifications

Although nature can produce a large number of metabolic alterations in the vitamin D molecule, organic chemists have vastly expanded the repertoire. A major incentive for this effort is to produce compounds with selectivity for one type of tissue or disease process that can be exploited clinically. Table 1 summarizes data from studies (4, 13–40) with a few of these analogs chosen either to illustrate certain points regarding structure-function relationships or to indicate trends in the clinical potential for these compounds. Figure 2 shows the structure of these analogs. In contrast to the earlier studies of metabolites and analogs summarized in the preceding paragraph and gathered during the 1970s, recent studies with newer analogs have taken advantage of models for the newly recognized actions of vitamin D. In particular, analogs have been sought that decrease proliferation and enhance differentiation of tumor cell lines and normal cells *in vitro* without increasing intestinal calcium absorption, serum calcium, bone resorption, or renal calcium excretion *in vivo*. Examples of tumor cell lines evaluated include HL-60 (a human promyelocytic leukemia cell line), U937 (a human promonocytic cell line), WEHI-3 (a mouse myelomonocytic leukemia cell line), and ROS 17/2.8 (a rat osteosarcoma cell line); examples of normal cells evaluated include keratinocytes, lymphocytes, and bone cells.

C. Importance of binding affinities

In interpreting the results of *in vitro* and *in vivo* studies, the importance of the different affinities of the analogs for both the cellular VDR and the serum DBP must be borne in mind. Thus, compounds such as 24,24-dihomo calcitriol (MC1147), 16 ene, 23 yne calcitriol (16ene, 23yne CT), 22 oxa calcitriol (OCT), and calcipotriol (MC903) have an affinity for the VDR that is within 1 order of magnitude of the affinity of calcitriol (CT). Yet these compounds are much less tightly bound to DBP. This produces several complicating results. *In vitro*, most cells are grown and studied in the presence of serum that contains DBP. We (41) have shown that the free CT concentration in culture media containing 10% fetal bovine serum is approximately 0.5% of the total concentration, and that the keratinocyte senses the free, not the total, concentration. These results have been confirmed by Bouillon *et al.* (13). They demonstrated that the ability of CT to inhibit the proliferation of phytohemagglutinin (PHA)-activated lymphocytes was inhibited 100-fold by the restoration of the normal concentration of DBP to DBP-depleted serum used at 10% concentration in the media for the experiment. In contrast, 24,24 dihomo CT and calcipotriol, which are much less tightly bound to DBP than CT, were proportionately much less affected by the presence of DBP. Thus, in evaluating the potency of a compound *in vitro* one must compare the free concentrations, not the total

TABLE 1. Structure-function relationships of vitamin D analogs

Compound	In vivo		In vitro	Antiproliferation			Prodifferentiation			Binding	
	ICA	SCa	Bone	Immune	Skin	Bone	Immune	Skin	Bone	VDR	DBP
1α, 25(OH)$_2$D$_3$	100	100		100 (13), (1) (13)	100	100	100	100	100	100	100
Shortening											
24 nor 1,25D$_3$	0 (4)	0 (4)					8 (34)				
26,27 bis nor 1,25D$_3$							1 (34)				
Lengthening											
24 homo 1,25D$_3$	100 (23, 28)	0 (23), 20–30 (28, 32)	100 (22)	200 (32)			100–800 (23, 28) (32, 34)			30 (23, 28)	
26 homo 1,25D$_3$	100 (28)	300 (28)	100 (22)				300 (28)–800 (34)			100 (28)	
24,24 dihomo 1,25D$_3$	1 (13)–10 (23)	0 (13)–4 (13, 23, 32)	0 (22)	3 (13, 13), 200 (32)			70–300 (13, 23, 3) (2)			3–25 (13, 23)	0 (13)–1 (16)
24,24,24 trihomo 22 Δ.1,25D$_3$	0 (23)	0 (23)	0 (22)				30 (23)			1 (23)	0.03 (16)
Stabilizing											
24,24F2 1,25D$_3$	300–1000 (35, 36)	100–300 (35, 36)			100		400–700 (37)	300		100 (14, 37)	
Other											
16 ene, 23 yne 1,25D$_3$	0 (13), 3 (24, 26), 53 (39)	0 (1), 2 (24, 26, 39)		400 (24)–1200 (26)	100		200–1000 (13, 2) (4, 26)	300		45–300 (13, 14, 1) (7, 24, 26)	0–0.2 (13, 16)
22 oxa 1,25D$_3$	<1 (33)	1 (29, 30, 33)	2 (31)	100 (15)–1000 (31, 40)			100 (15)–1000 (31, 39, 40)		100 (19, 21)	10 (15)–100 (19, 21, 40)	0.2–0.4 (16, 40)
Calcipotriol	<1 (13)	3 (13, 27)	10–30 (18)	30 (13), 100 (27)	100 (38)	100 (20)	100 (13, 27)	10–100 (38)	100 (20), 1–10 (25)	60–300 (13, 18, 27)	2 (16)

Underlined references indicate samples studied in presence of serum, generally 2 to 10%. ICA, Intestinal calcium absorption; SCa, serum calcium.

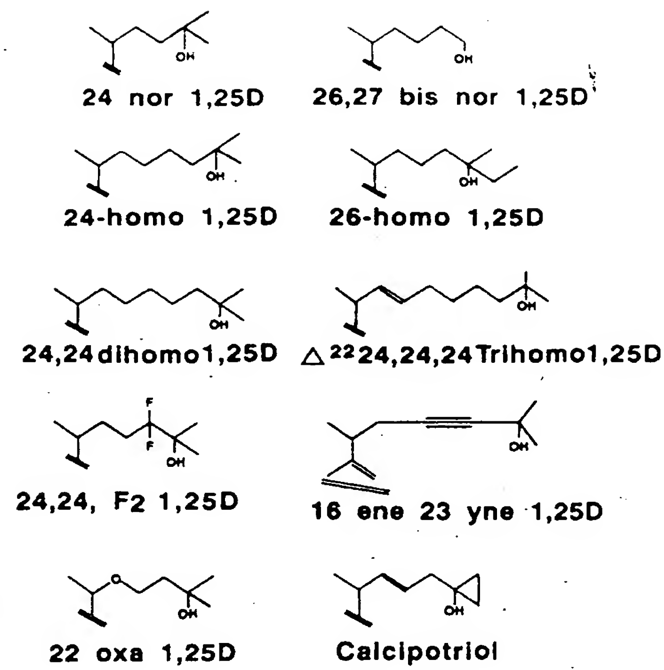

FIG. 2. Side chain modifications of CT. The analogs shown are those listed in Table 1 and discussed in the text.

concentrations. In Table 1, I have indicated which studies were performed in the presence of DBP containing serum. Nearly all *in vitro* cell culture studies have been performed in this fashion, the exceptions being the aforementioned study of human lymphocytes by Bouillon *et al.* (13), our own unpublished studies with human keratinocytes (Bikle, D., and Pillai, S., in preparation), and the study of human bone cells by Marie *et al.* (25). Except for 16ene, 23yne CT, which has a higher affinity than CT for the VDR at least in keratinocytes (14), and 24,24-difluoro-CT (24,24 F_2 CT) which may be more slowly catabolized than CT *in vitro*, much of the apparent increase in potency of the analogs relative to CT in inhibiting proliferation and stimulating differentiation *in vitro* is due to their higher free fraction in media containing serum. The different affinities of the analogs for DBP also affect the interpretation of *in vivo* studies. As observed by Dusso *et al.* (16), OCT has a shorter half-life and higher metabolic clearance rate *in vivo* than does CT. Other analogs with little affinity for DBP are likely to show similar short half-lives *in vivo*. The rapid clearance of these analogs *in vivo* may account for at least part of their selective effects. For example, raising serum calcium levels through changes in intestinal calcium transport and bone resorption where new protein synthesis may be required (42, 43) conceivably requires a more sustained level of circulating CT than inhibition of PTH synthesis or secretion (44, 45). Thus, the ability of

OCT to inhibit PTH secretion *in vivo* without raising serum calcium levels (30) could be due to this mechanism. Furthermore, these pharmacokinetic considerations may explain why CT appears to have a greater effect on bone resorption *in vitro* (18) than would be indicated by its limited effect on serum calcium levels *in vivo* (13, 27).

D. Genomic and nongenomic responses

Although differences in the relative affinities of DBP and VDR for the various analogs compared to CT can explain part of the selectivity observed for some of the analogs, differences in the manner in which different cells respond to CT and its analogs must also be considered. Not all vitamin D-regulated events require the interaction of the VDR-hormone complex with the genome to induce or inhibit new protein synthesis. This is illustrated by the cycloheximide-insensitive vitamin D-induced changes in calcium transport across the intestinal brush border (42, 46–48) and the vitamin D-induced rapid changes in intracellular calcium and/or phosphoinositide metabolism in cells from the intestine (49), liver (50), bone (51), and skin (52). This point becomes relevant to understanding the observation by Farach-Carson *et al.* (17) that the rank order of effectiveness for analogs in increasing calcium influx into ROS 17/2,8 cells does not correlate with the rank order of the affinity of the analogs for the VDR. Thus, it appears that analogs may differ in their ability to influence genomic and nongenomic mechanisms, and that the relative importance of genomic and nongenomic mechanisms in responding to the vitamin D metabolite or analog will contribute to the selectivity of that molecule.

E. Structure-function studies

A striking feature of the analogs listed in Table 1 is the profound influence caused by modest changes in the side chain. Shortening the side chain by one carbon (*e.g.* 24 nor CT) essentially eliminates its potency *in vivo* (4) and reduces its effectiveness *in vitro* by 1 order of magnitude (34). Removing two or more carbons from the side chain (*e.g.* 26, 27 bis nor CT) reduces its *in vitro* activity another order of magnitude (34). In contrast, lengthening the side chain by one carbon (*e.g.* 24 or 26 homo CT) preserves *in vitro* activity and the ability to stimulate intestinal calcium transport (23, 28, 32, 34). However, bone mobilization as assessed *in vivo* is less stimulated by 24 homo CT and more stimulated by 26 homo CT than would be expected from the comparable abilities of these analogs to stimulate bone resorption *in vitro* (22, 23, 28, 32). Addition of two carbons to the side chain (*e.g.* 24, 24 dihomo CT or MC1147) maintains the ability of this analog to inhibit lymphocyte proliferation and stimulate HL60 and U937 differentiation *in vitro* (13, 23, 32) while decreasing its ability to stimulate bone resorption *in vitro* (22) or increase intestinal calcium transport and serum calcium *in vivo* (13, 23). Adding a third carbon to the side chain (*e.g.* 24, 24, 24 trihomo 22ene CT) essentially abolishes *in vivo* activity with only a modest reduction in the ability to stimulate HL60 differentiation (22, 23). Substituting fluoride for hydrogen in those sites of the CT molecule that undergo further metabolism (*e.g.* C24, C26, C27) retards metabolism at those sites and is expected to increase the biological half-life of the molecule, although this has not been conclusively demonstrated. As exemplified by 24,24 F_2 CT, the fluoride substitution increases the *in vitro* and *in vivo* effects of CT without altering its ability to bind to the VDR (14, 35–37). This analog may be useful for conditions in which a sustained CT effect is desired.

In contrast to 24,24 F_2 CT, but similar to 24,24 dihomo CT, the remaining three analogs listed in Table 1, namely 16ene, 23yne CT, OCT, and calcipotriol, all have limited hypercalcemic effects *in vivo* (13, 24, 26, 27, 29, 30, 33, 39), yet are potent analogs in terms of their antiproliferative and prodifferentiating effects *in vitro* (13–15, 19–21, 24, 26, 27, 31, 37–40). Although some of the difference between *in vivo* and *in vitro* effects may reflect the rapid clearance of these analogs *in vivo*, this is not the total explanation. Abe *et al.* (31) noted that OCT was several orders of magnitude less potent than CT in stimulating bone resorption *in vitro*, while approximately 1 order of magnitude more potent than CT in inhibiting proliferation and stimulating differentiation of WEHI-3 cells. Furthermore, OCT appears to be 2 orders of magnitude more potent than CT in stimulating the immune response of mice to sheep erythrocytes *in vivo* (29), and equivalent to CT in suppressing PTH secretion when administered *in vivo* (30) despite its lack of effect on serum calcium levels. An important potential difference in the mechanisms of action between OCT and CT is that OCT failed to raise the intracellular free calcium levels (Cai) in HL60 cells or enhance the fMLP-induced Cai spike and superoxide generation. Yet OCT had effects comparable to CT on proliferation and differentiation of this cell line (15). Similarly, the CT-induced increase in Cai in ROS 17/2.8 cells could not be reproduced by OCT (51). Unlike the slow response of Cai to CT in HL60 cells, the response of Cai to CT in ROS cells is acute, suggesting that the mechanisms differ between the two cell lines, yet OCT apparently fails to activate the responsible mechanism in either case. It remains to be determined whether other analogs will share this potentially important difference in mechanism of action between CT and OCT on Cai, whether this difference will be found in all target cells, and whether this difference contributes to the selectivity of OCT for the parathyroid gland and the immune system *in vivo*.

However, such differences in fundamental mechanisms of action and the pharmacokinetic differences related to differential binding to DBP and VDR indicate that vitamin D analogs can be made with selective biological properties that can be exploited therapeutically. Several of these are already in clinical trials as will be described below.

III. Clinical Applications

The potential new clinical applications that will be discussed in this section are listed in Table 2. For some, such as the use of CT (or its analogs) in the management of osteoporosis, hyperparathyroidism accompanying renal failure, or psoriasis, existing data from clinical trials are quite promising. For others, such as the treatment of immune disorders, malignancy, hypertension, or diabetes mellitus, the use of CT or its analogs appears more distant.

The newly discovered actions of CT relevant to its new therapeutic potential can be considered of two sorts: 1) modulation of hormone and cytokine production and secretion, and 2) regulation of proliferation and differentiation. CT may exert its influence on cells by actions in one or both categories. An attempt to illustrate these points is shown in Figs. 3 and 4. In Fig. 3, CT is depicted as having a positive effect on insulin secretion by the B cell of the pancreas but a negative effect on PTH secretion from the parathyroid gland. In turn, both insulin and PTH stimulate CT production by the kidney. Such experiments lead to the possible role of CT in the management of diabetes mellitus and hyperparathyroidism. In Fig. 4 CT is depicted as having an antiproliferative effect on tumor cells and basal cells of the epidermis while promoting their differentiation as well as the differentiation of the bone cell precursors for both osteoblasts and osteoclasts. Most likely these effects on proliferation and differentiation involve regulation of, or at least interaction with, a number of cytokines (and hormones) in the various tissues that participate in the control of proliferation and differentiation of these tissues. Such actions suggest the usefulness of CT or its analogs in the treatment of osteoporosis, osteopetrosis, cancer, immune disorders, and psoriasis. These two recurring themes, the ability of CT to regulate hormone and cytokine secretion and its potential to modulate growth and differentiation of its "nonclassical" target tissues, underlie the development of CT and its analogs for new clinical indications.

A. Metabolic bone disease

1. Background. Although the use of vitamin D and its metabolites for the management of certain metabolic bone diseases is well established, the new understanding of the role of CT in the differentiation of osteoclasts and osteoblasts offers a rationale for the use of CT and its analogs in the management of osteoporosis and osteopetrosis. Furthermore, the discovery of the VDR in parathyroid tissue and the elucidation of its role in regulating PTH synthesis have led to a new approach in the management of primary and secondary hyperparathyroidism. In the treatment of these different conditions, hypercalcemia and/or hypercalciuria frequently limit the amount of CT that can be employed, and suboptimal doses may be required and/or dietary calcium may need to be restricted. For this reason, CT analogs with less potential for hypercalcemia may play a more important role in these clinical applications.

The amount of bone in the skeleton is controlled by the balance of bone formation and bone resorption that are mediated by osteoblasts and osteoclasts, respectively. The osteoblast contains receptors for CT; the osteoclast does not (53). Osteoblasts are responsible for bone formation, but the role of vitamin D in this process is not clear. In cultured rat or mouse calvaria and the osteoblast-like cells derived from them, CT inhibits collagen synthesis and alkaline phosphatase at concentrations

TABLE 2. Potential new clinical applications for CT and its analogs

Disease	Postulated actions
Osteoporosis	Increase bone mineral absorption from gut
	Enhance osteoblast differentiation and function
Osteopetrosis	Enhance osteoclast differentiation
Hyperparathyroidism	Suppress PTH synthesis
Cancer	Decrease proliferation, enhance differentiation
Immune disorders	Enhance suppressed activity in vitamin D deficiency
	Reduce inflammatory response of activated cells
Hypertension	Reduce calcium accumulation by vascular smooth muscle
Diabetes mellitus	Increase insulin secretion
	Correct decreased calcitriol production in insulin deficiency
Psoriasis	Decrease inflammatory component
	Decrease proliferation, enhance differentiaton of epidermis

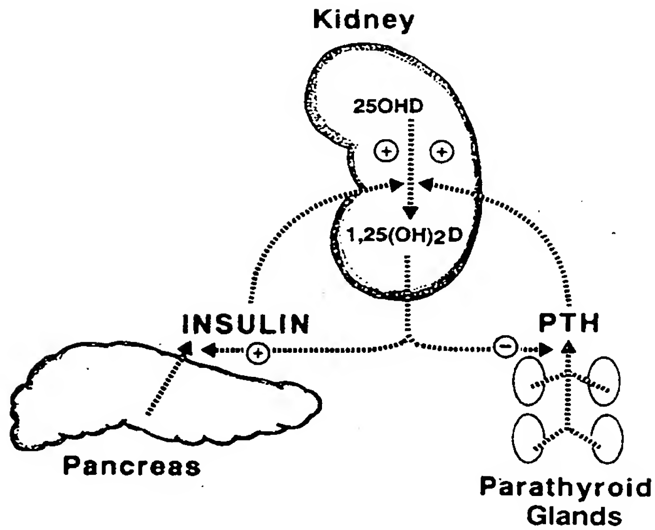

FIG. 3. Endocrine interactions of CT. This figure illustrates the rationale for the potential usefulness of CT in the management of diabetes mellitus and hyperparathyroidism. CT stimulates insulin secretion but inhibits PTH secretion. Both PTH and insulin increase CT production. In diabetes mellitus acute insulin deficiency may result in decreased CT production. Conversely, insulin secretion may be blunted in vitamin D deficiency and/or enhanced by CT supplementation. Hyperparathyroidism developing in patients with renal failure (and depressed CT production) responds to CT supplementation. Primary hyperparathyroidism may also be amenable to treatment with CT analogs that inhibit PTH secretion but do not aggravate the hypercalcemia.

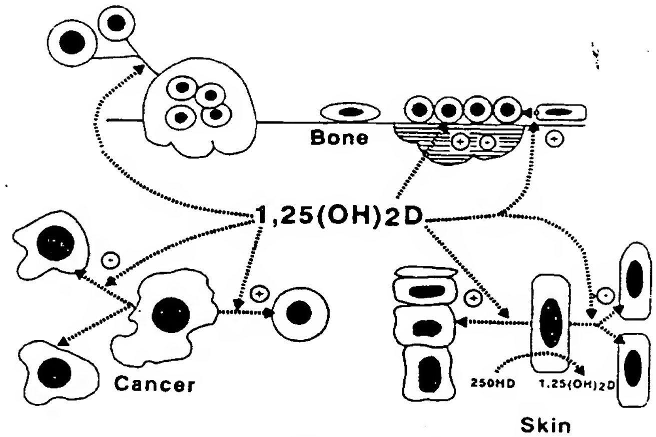

FIG. 4. Antiproliferative, prodifferentiating effects of CT. This figure illustrates the potential usefulness of CT in the management of selected bone disorders, malignancies, and hyperproliferative skin disorders. By promoting the differentiation of osteoblasts and osteoclasts, CT may be useful in the management of osteoporosis and osteopetrosis. Malignancies of cells containing the VDR may respond to both the antiproliferative and prodifferentiating effects of CT and its analogs. Psoriasis is already being treated successfully with CT and its analogs, with a decrease in the hyperproliferation and a change to a more normal appearing (*i.e.* better differentiated) skin. Note that in the epidermis, CT may have an autocrine or paracrine function because of the ability of keratinocytes to make this potent metabolite.

comparable to those required to stimulate bone resorption (54–57). In apparently less differentiated cells, such as the mouse osteoblastic cell line MC3T3-E1 and various human osteoblastic cell lines, CT stimulates both collagen synthesis and alkaline phosphatase (19, 58). This seeming paradox may be explicable by the ability of CT to promote the differentiation of the osteoblast (59), pushing osteoblast precursors to a more mature phenotype with higher bone-forming capabilities (reflected by increased collagen synthesis and alkaline phosphatase) while inhibiting these functions in the mature cell. This may be part of the mechanism by which CT stimulates bone formation. However, the ability of calcium and phosphate supplementation alone to reverse vitamin D-deficient rickets (60) or rickets due to a dysfunctional VDR [hereditary vitamin D-resistant rickets or vitamin D-dependent rickets type II (VDDR II)] (61, 62) suggests that the direct effect of CT on the osteoblast is not essential for bone formation. The differentiated osteoblast is required for $1,25(OH)_2D$-stimulated bone resorption acting as the transducer for CT to activate the osteoclast presumably by the release of cytokines that can act on the osteoclast (63). Osteoclasts do not have the VDR and do not respond to CT directly (63). However, as for osteoblasts, CT is an important regulator of osteoclast differentiation, promoting their formation from hematogenous precursors that do contain the VDR (64). Thus, CT is likely to play an important role in bone remodeling not only by its ability to provide adequate calcium and phosphate for mineralization but by influencing cytokine secretion by osteoblasts and differentiation of bone cell precursors.

2. *Osteoporosis.* Numerous studies attempting to treat postmenopausal osteoporosis with vitamin D and its metabolites have been performed, and the results have been mixed. Three recent studies (65–67) using the same protocol in which CT was administered over a 2-yr period and the dose adjusted to avoid hypercalcemia reported somewhat different results. The study by Ott and Chestnut (66) used the lowest mean dose of the three studies and showed no effect on histomorphometric measurements or on cortical bone density measured by single or dual photon absorptiometry techniques. Trabecular bone density measured by quantitative computed tomography of the spine appeared to increase, although the number of patients serially studied by quantitative computed tomography was small. In contrast, the studies by Aloia *et al.* (65) and Gallagher and Goldgar (67) showed significant increases in spinal bone density and total body calcium compared to placebo-treated controls. None of these three studies showed a significant decrease in frac-

ture rate, and hypercalcemia and hypercalciuria complicated treatment in some of the subjects. The considerably larger 3-yr study by Tilyard *et al.* (68) evaluated 314 women treated with a twice daily dose of 0.25 μg CT compared to 308 women treated with 1 g calcium supplementation. They observed a 70% reduction in the incidence of new fractures after the first year of treatment with only two cases of hypercalcemia and one case of decreasing renal function. Likewise, an up to 8 yr study by Caniggia *et al.* (69) of 270 postmenopausal osteoporotic women treated with 1 μg CT/day without calcium supplementation showed a 75% reduction in fractures after the first year. This was accomplished without an increase in serum calcium or deterioration of renal function. However, the subjects treated with CT in these studies did have a significant increase in urinary calcium excretion. Similar results have been obtained with the CT analog, 1α-hydroxyvitamin D (1αOHD) (70, 71). Conceivably, analogs with less potential for increasing serum calcium relative to their effects on bone could provide a safer alternative to CT in the management of osteoporosis allowing for larger doses and less intense monitoring.

3. Osteopetrosis. Osteopetrosis is caused by a failure to resorb bone and is due to impaired osteoclast function most likely of several etiologies including impaired cytokine production (72, 73). Bone marrow transplant may be curative (74), attesting to the hematogenous origin of the osteoclast. In a recent evaluation of 16 infants with malignant osteopetrosis, Cournot *et al.* (75) found that many had low or low normal serum calcium and phosphate levels accompanied by increased alkaline phosphatase, PTH, and 1,25(OH)$_2$D levels suggesting resistance to the bone-resorbing effects of these hormones. Histological observations of the bone from six subjects revealed abundant osteoclasts in five but none in one, whereas osteoblasts were reduced in all. Although it is not clear to what extent CT can correct the abnormal function of these bone cells, CT has been used in very high doses to enhance bone resorption *in vivo* and *in vitro* with monocytes from one such patient (76). In this latter study a low calcium diet was employed to mitigate the hypercalcemia.

4. Hyperparathyroidism. PTH synthesis and secretion are inhibited by both calcium and CT (77). The parathyroid gland contains VDR (78, 79), which are reduced in uremia (80, 81), potentially making the parathyroid gland less sensitive to CT. Similarly, the response of the parathyroid gland to calcium is blunted in uremia, but the mechanism is unclear (82). CT inhibits PTH secretion by inhibiting its synthesis at the level of gene transcription (44, 83), and a vitamin D response element has been identified in the 5′-flanking region of the PTH gene (84). The response of PTH secretion to CT requires hours. Unlike CT, calcium exerts a direct effect on PTH secretion, an effect seen rapidly and thought to be mediated by a calcium response element in or near the plasma membrane (85). However, calcium also reduces the messenger RNA levels for prepro-PTH indicating that it also exerts an effect on synthesis either at the level of transcription or message stability (86). Thus, nonhypercalcemic analogs of CT could be useful in the management of this condition.

5. Chronic renal failure. Secondary hyperparathyroidism complicates chronic renal failure, often occurring before serum calcium levels fall but generally in association with decreased 1,25(OH)$_2$D levels (87). It has long been appreciated that oral CT administration to patients with renal failure raises their serum calcium level, reduces their PTH secretion, and improves their clinical condition (88, 89). At least part of this effect is due to the increase in intestinal calcium absorption with CT therapy. Unfortunately, many patients with severe hyperparathyroidism are quite sensitive to CT in that they develop hypercalcemia with doses that have little impact on their PTH levels. Slatopolsky *et al.* (90) observed that the iv administration of CT reduced PTH levels more effectively and with less increment in serum calcium than oral administration. Presumably this is due to the higher levels of CT that can be achieved systemically with less exposure of the intestinal epithelium when the iv route is used. A subsequent study by Andress *et al.* (91) indicated that iv administration of CT could be used to correct the biochemical and skeletal abnormalities of secondary hyperparathyroidism in patients who could not be adequately managed with oral CT. Delmez *et al.* (92) confirmed the observation that iv CT could reduce PTH levels without raising serum calcium, and by manipulating the serum calcium with calcium infusions or low calcium dialysate baths showed that CT increased the sensitivity of the parathyroid gland to inhibition by calcium. That is, CT reset the set point to a lower calcium concentration. Similar data were obtained by Dunlay *et al.* (93). Since 1αOHD requires 25 hydroxylation (presumably only in the liver in humans) to be active, it and the nonhypercalcemic analogs under recent development may provide oral alternatives to iv CT in the management of secondary hyperparathyroidism. These studies indicate that the secondary hyperparathyroidism of chronic renal failure will be one of the first new indications for treatment with CT or one of its analogs.

6. Hypophosphatemic rickets. Patients with X-linked hypophosphatemic rickets have an abnormality not only in phosphate handling but in 1,25(OH)$_2$D production (94–97). Their calcium levels tend to be in the low normal range. When treated with vitamin D and phosphate,

hyperparathyroidism often develops, although serum calcium levels remain normal (98, 99). This may be due to further reduction in $1,25(OH)_2D$ production as a result of the phosphate therapy. CT more effectively heals the bone disease and affords better control of PTH than does vitamin D (97, 99). However, hyperparathyroidism can persist, although its impact on bone as these children grow into adults is not clear. Whether more aggressive treatment with CT or one of its nonhypercalcemic analogs should be initiated in patients with persistent hyperparathyroidism remains uncertain.

Although iv administration of CT represents an important advance in the treatment of secondary hyperparathyroidism complicating the management of renal failure and has been used successfully in the treatment of primary hyperparathyroidism (100), patients treated chronically in this fashion do develop increased serum levels of calcium and phosphate that may limit treatment (89). Therefore, nonhypercalcemic analogs are being evaluated. Brown *et al.* (30) showed that OCT, like CT, inhibited PTH secretion and reduced its gene transcription *in vitro* without raising serum calcium *in vivo*. As yet no clinical trials with OCT or the other nonhypercalcemic analogs have been performed to demonstrate their ability to inhibit PTH secretion *in vivo*.

B. Cancer

1. Epidemiology. Garland *et al.* have recently reviewed evidence correlating calcium and vitamin D with colon (101) and breast (102) cancer. Of 15 cancers evaluated, only these two showed a negative correlation between cancer incidence and the ambient UV light intensity when data from 87 locations throughout the United States were compiled. UV light exposure is suggested as a measure of cutaneous vitamin D production. In a large prospective study, these investigators noted a negative correlation between dietary calcium, vitamin D, serum 25OHD, and the incidence of colon cancer (103, 104). Similar data have been obtained at least for dietary calcium and colon cancer by others (105–107), although the correlation between vitamin D deficiency and breast cancer has been challenged (108). Schwartz and Hulka (109) have suggested that mortality from prostate cancer might also be linked to vitamin D because of a geographic relationship between mortality from this malignancy and UV light intensity similar to that for breast and colon cancer.

2. Laboratory and animal studies. Eisman *et al.* (110) detected VDR in breast cancer lines more than a decade ago, and the list has rapidly expanded to include a wide variety of malignancies from lung, cervix, bone, skin, colon, lymphatic, and hematopoietic tissue (111). In general, $1,25(OH)_2D$ inhibits the proliferation and stimulates the differentiation of these cell lines *in vitro* (112–121). Furthermore, vitamin D, $1,25(OH)_2D$, or its analog 1α OHD have been shown to decrease tumor size, number, or lethality when given *in vivo* to animals in which the tumors were chemically induced (122–125) or grafted (126–128). Although a dose-response relationship between the vitamin D compound and its anticancer effect has been demonstrated *in vivo* and *in vitro*, the dose *in vivo* is restricted by toxicity. Thus, optimally effective doses in terms of preventing tumor growth increase mortality presumably by inducing hypercalcemia (125). Similar constraints limit the use of CT in the treatment of malignancies in humans (129), and as of yet no compelling study has shown its usefulness for this purpose in humans. However, OCT has recently been shown to inhibit the proliferation of human breast cells *in vitro* at doses 10–100 times less than CT and *in vivo* in mice without causing hypercalcemia (130). Likewise, 16ene, 23yne CT has been shown in mice to be more effective than comparable doses of CT in increasing survival after the injection of leukemic cells and does so without inducing hypercalcemia (131). Thus, the availability of the nonhypercalcemic analogs of CT should permit a reexploration of the role of vitamin D in the management of malignant disease.

3. Tumor production of $1,25(OH)_2D$. Most malignancies cause hypercalcemia either by direct effects on bone (through elaboration of cytokines that induce bone resorption) or by elaborating PTH-related peptide. However, in a small number of Hodgkin and non-Hodgkin lymphomas (which except for HTLV-1 T-cell leukemia/lymphoma are seldom associated with hypercalcemia), the hypercalcemia appeared to be due to increased CT synthesis by the tumor (132–142). In one case (142) this was confirmed *in vitro*. Chemotherapy including glucocorticoids is used to treat these tumors, and successful treatment corrects both the elevated calcium and CT levels. In one report (140), recurrence of the Hodgkins disease was associated with recurrence of hypercalcemia and increased CT levels. However, it has not been established that production of $1,25(OH)_2D$ by the lymphoma correlates with degree of differentiation or prognosis. One possibility is that as in sarcoidosis and other granulomatous diseases, the $1,25(OH)_2D$ production occurs in abnormal macrophages within the tumor which are not subject to normal feedback inhibition or are bathed in cytokines such as interferon-γ (IFN-γ) which promote $1,25(OH)_2D$ production. This aspect will be discussed in the section dealing with vitamin D and the immune system.

C. Immune function

1. Cellular effects of CT. Substantial evidence is accumulating that CT plays an important modulatory role in

the immune system (Fig. 5). Peripheral blood mononuclear cells acquire VDR when they are activated *in vitro* by agents such as PHA (143, 144). Activation of such cells leads to proliferation and elaboration of a variety of cytokines. CT inhibits proliferation (at the level of G1) and the production of IL-2, IFN-γ, and granulocyte macrophage colony stimulating factor by PHA-activated peripheral blood mononuclear cells (145–147). Studies on the effects of CT on interleukin-1 (IL-1) and tumor necrosis factor production have shown both stimulation and inhibition (148–151). CT stimulates H_2O_2 production in macrophages (152), monocyte adherence, and through the induction of heat shock proteins may protect the cell during the febrile response (153). *In vitro*, immunoglobulin production is depressed by CT (144, 154, 155), an effect that appears to be mediated by an inhibitory action on the helper function of T cells rather than a direct effect on B cells (144). Although the effects of CT on the immune functions of T cells are predominantly inhibitory when the cells are activated by PHA, CT is less inhibitory and may even be stimulatory when the cells are activated by phorbol esters or the anti-T3 monoclonal antibody OKT3 (149). Thus, the mechanism by which the immune system is activated could determine the degree or even the direction of immunomodulation by CT.

2. Production of CT by macrophages. The effects of CT on these immune functions may represent a paracrine or autocrine action. Activated (as by IFN-γ or lipopolysaccharide) normal macrophages make CT (156, 157), as do macrophages from granulomatous diseases such as sarcoidosis (158, 159) and tuberculosis (160, 161). Conceivably, the increased production of CT in such disease

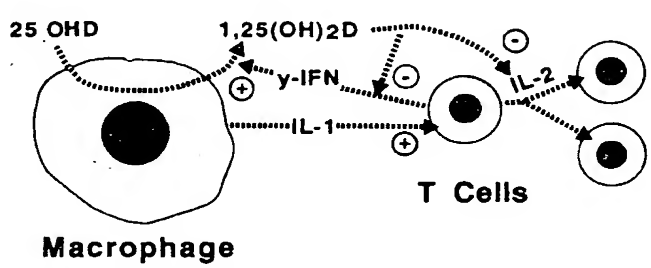

FIG. 5. Selected effects of CT on immune function. CT is made by activated macrophages and could serve to suppress the proliferation of lymphocytes by direct inhibition of the cell cycle as well as by inhibition of IL-2 secretion. Interferon-γ stimulates CT production by the macrophage, and its secretion by the lymphocyte is in turn inhibited by CT. Not shown here is that T cell activation of B cell production of immunoglobulins is also inhibited. These suppressive effects of CT on the immune system suggest that CT or its analogs may be useful in the management of inflammatory states including autoimmune disorders. However, vitamin D deficiency has been associated with decreased resistance to certain infections suggesting that CT has more than an immunosuppressive role.

states serves a protective function, but it may be sufficiently extensive that hypercalcemia ensues.

3. Clinical studies. Although much of our knowledge about CT-regulated immune function stems from *in vitro* studies, immune dysfunction in vitamin D-deficient or -resistant states has been observed indicating the clinical importance of these *in vitro* observations. Nutritional vitamin D deficiency is associated with increased risk of infection, and the neutrophils from such children appear to have abnormal motility and phagocytic ability (162, 163). Vitamin D deficiency has also been reported to predispose to disseminated tuberculosis (164). Toss and Symreng (165) reported a correlation between low 25OHD levels and anergy to skin testing in 63 elderly subjects; vitamin D treatment of five anergic subjects with low 25OHD levels normalized both the skin reactivity and the 25OHD level. Patients with chronic renal failure requiring hemodialysis were found to have reduced lymphocyte responsiveness to the proliferative stimulus of lectins, which was normalized by 4 weeks of 1αOHD administration (166). Walka *et al.* (167) described a patient with VDDR II who had myelofibrosis and recurrent septicemia, and who on further investigation had abnormal neutrophil chemotaxis and an inability to produce immunoglobulin G antibodies to lipid A after each episode of septicemia. Calcium infusions corrected the myelofibrosis and the rickets but not the immunological abnormalities. Etzioni *et al.* (168) described five patients with VDDR II, four of whom had reduced white cell phagocytosis of *Candida albicans* and all of whom had decreased intracellular killing of this organism. Calcium infusion *in vivo* did not correct these abnormalities measured *in vitro*, although the calcium ionophore ionomycin corrected the *in vitro* defect in cell killing suggesting that CT may stimulate cell killing, at least in part, by raising intracellular calcium. This is of interest since the analog OCT does not raise intracellular calcium to the same degree as CT at least in HL-60 cells (49). Kitajima *et al.* (169) reported six cases with hypophosphatemic vitamin D-resistant rickets who had frequent infections (colds, cystitis, bronchitis, and pneumonia) that appeared to improve after 1αOHD treatment. These patients had decreased numbers of natural killer cells and increased levels of leukocyte adenosine deaminase that normalized with treatment. On the other hand, a hyperfunctioning immune system as in chronic inflammatory conditions might also be treated with CT in vitamin D-replete individuals as suggested by the reduction in joint pain observed in seven of ten patients with psoriatic arthritis treated with CT (170).

4. Animal studies. Animal studies support these clinical observations and suggest a role for CT or its analogs in the management of immune disorders. Stroder (171)

reported a reduction in antibody formation in ricketic rats challenged with diphtheria toxin or Sendai virus, a more fulminant spread of pneumococcal and *Klebsiella* infection when ricketic rats were inoculated with these organisms, and a decrease in antibody forming cells in the spleen from ricketic rats. Bar-Shavit *et al.* (172) noted reduced chemotaxis and phagocytosis of peritoneal macrophages from vitamin D-deficient mice which could be corrected by vitamin D treatment. CT blocked the development of murine experimental autoimmune encephalitis (173), whereas the CT analogs OCT and $1,24(OH)_2D$ blocked the development of renal disease and improved survival in the MRL mouse model of autoimmunity (174, 175).

In sum, not only is vitamin D deficiency or resistance associated with subtle abnormalities in the immune system that predispose to infection, but the immunomodulatory actions of CT or its nonhypercalcemic analogs may prove to be useful agents in controlling the immune system when it goes awry. Clinical studies testing this possibility need to be performed.

D. Hypertension

1. Role of calcium. Considerable interest in the relationship between calcium and hypertension has developed over the past decade. Calcium is clearly necessary for the contractile response in all forms of muscle including the heart and vascular smooth muscle. Calcium channel blockers are effective means of controlling hypertension, presumably by limiting the influx of calcium into the cells responsible for maintenance of vascular tone. Thus, increased serum calcium or hormones such as PTH and CT might be expected to lead to hypertension by raising intracellular calcium concentration in the vascular smooth muscle. Consistent with this view is the observation by Erne *et al.* (176) that the intracellular free calcium concentration (Cai) of platelets correlates positively with the degree of hypertension, the positive correlation between serum calcium and blood pressure observed by Kesteloot and Geboers in 9000 young males (177), the association between hyperparathyroidism and hypertension (178, 179) and between CT levels and blood pressure (180), the increase in blood pressure induced by prolonged PTH infusions (181) (although acute PTH infusions are vasodilatory (182, 183), and the increase in blood pressure and peripheral vascular resistance seen acutely after calcium infusion (184–188). Calcium channel blockers used in the treatment of hypertension reduced platelet Cai (176) and prevented the acute increase in blood pressure during calcium infusion (185).

Although the link between calcium and hypertension seems clear, McCarron and Morris (189) have argued that it is calcium deficiency rather than excess that leads to essential hypertension. A large number of epidemiological studies support the claim that dietary calcium deficiency and reduced serum calcium levels are associated with hypertension (reviewed in Ref. 189), and dietary calcium supplementation exerts a modest blood pressure-lowering effect in some patients especially those with the highest blood pressure and lowest serum calcium level (190–194). Renal calcium wasting has been described in patients with essential hypertension (195, 196), as has decreased intestinal calcium absorption by some (197, 198), but not all (199), groups evaluating the spontaneously hypertensive rat. Resnick and his colleagues have stratified their hypertensive patients according to renin levels. They demonstrated that the low renin group is more likely to have low serum calcium, elevated PTH and CT levels (200), and respond best to calcium supplementation (201) or calcium channel blockers (202).

2. Role of vitamin D. Thus, an apparent paradox exists between the presumed increase in Cai in the vascular smooth muscle and the presumed deficiency of calcium and lower serum calcium levels in hypertensive patients. In the study (176) demonstrating the increased platelet Cai in hypertensive patients, the authors also noted decreased serum ionized calcium levels, confirming this apparent paradox. Therefore, altered calcium handling by the cell has been implicated in the pathogenesis of hypertension (189), possibly at the level of reduced CaATPase activity (203), leading to increased Cai but decreased serum calcium due to decreased intestinal calcium absorption and renal calcium wasting (189). Calcium flux is regulated by CT in a number of tissues. VDR have been demonstrated in both heart (204) and vascular smooth muscle (205) cells. CT stimulates calcium flux at least in heart cells (206). Vitamin D deficiency is associated with increased contractility of both the heart and vascular smooth muscle (207, 208) suggesting increased Cai despite reduced serum calcium levels. Consistent with these findings are the observations by Baksi (209) that rats raised on either a vitamin D-deficient or calcium-deficient diet have elevated blood pressures compared to controls on normal diets. At least one clinical study has suggested that 1αOHD could be used to treat hypertension (210).

Although these studies are provocative and suggest a role for CT and/or its analogs in the management of hypertension, considerable uncertainty remains regarding this potential application. However, if the apparent abnormality in calcium handling by cells (in particular, vascular smooth muscle cells) in hypertensive subjects is confirmed and shown to be corrected by CT, clinical trials with CT or its analogs could be initiated.

E. Diabetes mellitus

1. Calcitriol regulation of insulin secretion. The discovery that the vitamin D-dependent calcium binding protein (211–213) and the VDR (214–216) are found in the pancreas was paralleled by the discovery that vitamin D deficiency resulted in decreased insulin secretion in response to glucose or arginine (217). This abnormality could be corrected with CT. Since calcium is required for insulin secretion and since insulin secretion is blunted by starvation, the actual mechanism by which CT exerts its effects is not completely clear. Vitamin D repletion of an erstwhile vitamin D-deficient animal leads to improved nutrition and increased serum calcium levels, both of which could result in enhanced insulin secretion. The studies intended to resolve this issue have shown that pair feeding vitamin D-replete animals with vitamin D-deficient animals blunts, if not abolishes, the ability of CT to restore normal responsiveness of the islet to glucose (218–220). Thus, nutrition plays an important role, although it does not explain all the effects of CT. Raising the serum calcium level of the vitamin D-deficient rat to normal does not restore normal responsiveness of the islet to glucose (218–220). However, such dietary manipulation results in profound hypophosphatemia (219) which could itself blunt insulin secretion. Nevertheless, studies by Tanaka *et al.* (221) and Ozono *et al.* (222) demonstrated that normalization of serum calcium by diet partially corrected the defect in insulin secretion equivalent to that by CT when the rise in serum calcium was prevented by a low calcium diet. In their hands the combination of CT and calcium repletion led to the greatest degree of insulin secretion. This interaction between calcium and CT may explain the findings of Hochberg *et al.* (223) who studied six children with VDDR II. Of the five tested for insulin secretion during a glucose tolerance test, the three with the most blunted response had the lowest serum calcium levels. *In vitro* incubation of islets from vitamin D-deficient rats with CT or inclusion of CT in the islet perfusate did not restore normal insulin secretion (218). However, the response to CT *in vivo* can be seen as early as 3 h after administration (224), which is before a substantial increase in either food intake or serum calcium level. In sum, the data support a direct role of CT in promoting insulin secretion in addition to an indirect role through changes in nutrition and serum minerals.

2. Insulin regulation of CT production. Compounding the possibility that insulin secretion requires adequate levels of CT are the observations that acute insulin deficiency leads to decreased CT production (225, 226). Thus, insulin secretion and CT levels could fall rapidly and in parallel before the diabetes is recognized and treated. This may account for the observation that bone loss in insulin-dependent diabetics occurs early, and then stabilizes unless the diabetes is poorly controlled (227–232). In BB diabetic rats, reduced CT levels are associated with decreased DBP levels (233) such that the calculated "free" CT levels are normal or even elevated. Despite this, intestinal calcium absorption, intestinal calcium binding protein, and VDR are reduced as one would find in vitamin D deficiency. Whether this estimation of the biologically available CT accurately reflects the *in vivo* situation in these animals is not clear, but if so would suggest that vitamin D resistance might also complicate the diabetic state. Observations in other experimental animal models of diabetes mellitus have also shown lower CT levels (234, 235), acute decrements in intestinal calcium transport (236), and decreased intestinal calcium binding protein (237) as well as decrements in bone formation (238).

3. Diabetes and metabolic bone disease. The clinical implications of these animal studies are not clear. Diabetes mellitus is not a well known complication of vitamin D deficiency. As mentioned previously, hypocalcemic patients with VDDR II may have a subtle defect in insulin secretion (223), but diabetes mellitus in this group has not been described. Patients with uremia (which results in decreased CT levels) are known to have abnormal carbohydrate metabolism. Mak (239) demonstrated an improvement in insulin secretion in a group of uremic subjects given CT 2 h before the glucose challenge. Conversely, reduced CT levels have been described in some (240, 241), but not all (242), studies of diabetics. The pregnant diabetic and her fetus may be at greatest risk of reduced CT levels (240), and hypocalcemia in the infant of the diabetic mother is common (242). The impact of altered vitamin D metabolism in the nonpregnant diabetic is more difficult to ascertain. Intestinal calcium absorption appears to be normal (242–244). Decreased bone density in both adult and juvenile onset diabetics has been reported by a number of groups (227–232), and the risk of fractures may be increased in this patient group (245, 246). Thus, with our current state of knowledge it is not clear that CT or its analogs has a major role to play in the management of diabetes mellitus. However, the possibility that CT or one of its analogs could enhance insulin secretion in the type 2 diabetic or prevent the loss of bone at the onset of type 1 diabetes mellitus needs to be considered.

F. Psoriasis

1. Targets for CT action. Psoriasis may be among the first of the new clinical applications for CT and its analogs to gain widespread acceptance. Psoriasis involves both an inflammatory component with infiltration of neutrophils and T lymphocytes into the dermis and a

hyperproliferative component of the epidermis with poorly differentiating keratinocytes. Both processes represent potential targets for CT. As discussed under "immune function" CT inhibits the elaboration of cytokines from activated lymphocytes and blocks their proliferation. Thus, this component of the inflammatory process seen in psoriasis may be blocked by CT. As we (247) have recently reviewed in depth, CT is also a potent modulator of keratinocyte proliferation and differentiation. Keratinocytes make CT (248, 249), contain VDR (250–252), and respond to CT with a decrease in proliferation and an increase in differentiation (251, 253, 254). As such, the hyperproliferative response of the epidermis in psoriasis should also be amenable to treatment with CT.

2. Clinical studies. Realization of the potential usefulness of CT in the treatment of psoriasis emanated from a case report in which a woman being treated for osteoporosis with 1αOHD showed clearing of her psoriasis (255). This report was followed by a number of small open clinical trials with either 1αOHD, 1,24$(OH)_2$D (comparable to CT in *in vitro* potency), or CT given orally or applied topically (256–258). Although not carefully established, it appears that the response is dose dependent (259, 260). Oral administration tends to increased serum and urine calcium levels limiting the amount of CT that can be safely given at least by this route. Data regarding the topical administration of CT suggest that higher concentrations can be administered by this route (261), but dose limitations have not been established. Because of the potential toxicity related to the use of CT, studies began with the nonhypercalcemic analog, calcipotriol (MC903). In a dose-response study Kragballe (262) used up to 100 μg calcipotriol/g vehicle without detecting a change in serum calcium levels. This dose has been extended to 1.2 mg/g vehicle without evident toxicity (263), although maximal effectiveness was achieved at 50 μg/g in the Kragbelle study (262). These doses are 2 to 3 orders of magnitude higher than the preparations of CT, 1,24$(OH)_2$D, or 1αOHD used by others (256, 258, 260, 261). Using the 50 μg/g dose in a large multicenter study with 345 patients, Kragbelle *et al.* (264) demonstrated that calcipotriol was equivalent to, if not better than, betamethasone in the treatment of psoriasis. Over 80% of the patients had marked improvement or clearing of their lesions with calcipotriol. No changes in serum calcium were reported; the only side effect noted in a significant number of subjects was burning or itching at the site of application.

These results are quite promising. However, the long term safety of the nonhypercalcemic analogs has not been established. These drugs could inhibit the normal renal production of CT and so produce a situation in which the "classic" actions of CT are reduced (*i.e.* decreased intestinal calcium absorption). Patients with psoriasis are likely to require lifelong treatment with these CT analogs. Whether their use in high concentrations will lead to osteoporosis (reduced intestinal calcium absorption but enhanced osteoclast differentiation) or other disorders in the bone mineral homeostatic system remains for future investigation. Administering the drugs topically is likely to lead to fewer complications because the keratinocyte actively metabolizes CT (248) and is likely to do the same to the analogs thus limiting systemic exposure. Nevertheless, studies are required to investigate the long term effects of these analogs on bone mineral homeostasis before their use becomes widespread.

IV. Conclusions

An exciting new era has developed in the vitamin D field with the discovery of new target tissues, mechanisms of action, and selective analogs. In this review I have discussed the structure-function relationships of both naturally produced metabolites as well as synthetic analogs. The list was chosen not to be complete but to be illustrative. In the next few years many more analogs are likely to be available at least for research purposes. Of the available analogs, those that do not raise serum or urine calcium may be the most useful when applied to the new indications.

With the discoveries that the VDR is found in a wide (but not universal) range of tissues and that CT influences those tissues in a variety of ways comes the potential to use CT therapeutically in novel ways. The ability of CT to inhibit PTH secretion has already led to its use in the management of secondary hyperparathyroidism. Conceivably, primary hyperparathyroidism could also be treated with a nonhypercalcemic analog. The ability of CT to stimulate insulin secretion suggests the possibility that type 2 diabetics might in the future be benefitted by a CT analog. The potential for developing analogs with selective effects on osteoblast and osteoclast differentiation and function could lead to more effective use of these drugs in the management of osteoporosis and osteopetrosis. Because of the antiproliferative and prodifferentiating effect of CT on a number of cell lines including malignant cell lines, the nonhypercalcemic analogs offer an approach to the management of malignancy of those tumors that contain a VDR. Immunological disorders, including chronic inflammation, might also be managed by the CT analogs because of their potent immunomodulatory properties. Conceivably, analogs will be developed that will be selective for the different immune functions which they alter. As the abnormal cellular handling of calcium in patients with

essential hypertension becomes better defined, a role may emerge for CT or one of its analogs to modulate this process in a way that could be useful for treating this common condition. The ability of CT to modulate both the immune function and the differentiation of epidermal cells has already led to substantial success in the treatment of psoriasis.

As we enter this new era it is important to bear in mind that the analogs might be a two edged sword. By interfering with normal vitamin D metabolism and mechanisms of action of the natural metabolites, the use of high doses of the analogs could alter the bone mineral homeostatic system in a deleterious way. Such effects may not appear in short term studies when only serum and urine calcium levels are examined. These effects may be of little consequence in the management of life-threatening illnesses such as cancer but are of greater concern in the lifelong treatment of a disease such as psoriasis.

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